REMARKS

By this Amendment, Applicants have amended claim 134 and added new claim 155. Applicants maintain that the amendments made hereinabove do not raise any issue of new matter.

Accordingly, claims 134 to 155 are pending in the subject application.

Support for New Claims

The subject application is a continuation of U.S. Serial No. 10/346,853, filed January 17, 2003, which is a continuation of U.S. Serial No. 09/100,812, filed June 19, 1998, now U.S. Patent No. 6,573,099 B2, issued June 3, 2003, which claims priority of Australian Provisional Patent Application No. PP2492, filed March 20, 1998 (the "Priority Application"). The new claims are fully supported in the disclosure of the Priority Application.

Independent claim 134

a) "double-stranded DNA construct"

The amendment inserting "double-stranded DNA" in claim 134 is supported, *inter alia*, by the numerous examples of "double-stranded" synthetic genes that are replete in the Priority Application. Specifically, a number of genetic constructs are described on page 28, line 14, to page 39, line 22 of the Priority Application. The genetic constructs described are ultimately derived from a double-stranded DNA plasmid, such as pCR2.1. *See, e.g.*, page 27, lines 1 to 8 of the Priority

Application. Applicants attach hereto as **Exhibit 1** a map of plasmid pCR2.1, which is a commercially available starting plasmid for a number of the Examples. Additionally, the Priority Application describes blunt-ended fragments, which implies that they are double stranded and have the potential to have an overhang. *See, e.g.*, page 37, lines 6 to 7 of the Priority Application.

Independent claim 155

a) "producing an RNA molecule" and "transcribed"

Support for this language may be found, *inter alia*, at page 7, lines 5 to 6; and page 18, lines 27 to 28 of the Priority Application.

b) "capable of delaying, repressing or otherwise reducing the expression of a target gene"

Support for this language may be found, *inter alia*, at page 1A, lines 7 to 9 of the Priority Application.

c) "mammalian cell"

Support for this language may be found, *inter alia*, at page 22, lines 19 to 21 and page 26, line 5 and line 11 of the Priority Application.

d) "introducing into a cell"

Support for this language may be found, *inter alia*, at page 23, lines 9 to 15 of the Priority Application.

e) "double-stranded DNA construct"

The amendment inserting "double-stranded DNA" in claim 134 is supported, *inter alia*, by the numerous examples of "double-stranded" synthetic genes that are replete in the Priority Application. Specifically, a number of genetic constructs are described on page 28, line 14, to page 39, line 22 of the Priority Application. The genetic constructs described are ultimately derived from a double-stranded DNA plasmid, such as pCR2.1. *See, e.g.*, page 27, lines 1 to 8 of the Priority Application. Applicants attach hereto as **Exhibit 1** a map of plasmid pCR2.1, which is a commercially available starting plasmid for a number of the Examples. Additionally, the Priority Application describes blunt-ended fragments, which implies that they are double stranded and have the potential to have an overhang. *See, e.g.*, page 37, lines 6 to 7 of the Priority Application.

f) "synthetic gene"

Support for this language may be found, *inter alia*, at page 7, lines 1 to 3 of the Priority Application.

g) "operable in the cell"

Support for this language may be found, *inter alia*, at page 3, line 16 of the Priority Application.

h) "transcription termination sequence"

Support for this language may be found, *inter alia*, at page 22, lines 12 to 14 of the Priority Application.

i) "active in the cell"

Support for this language may be found, *inter alia*, at page 22, lines 19 to 20 of the Priority Application.

j) "operable connected thereto"

Support for this language may be found, *inter alia*, at page 11, lines 28 to 29 of the Priority Application.

k) "first and second structural gene sequences"

Support for this language may be found, *inter alia*, at page 3, lines 9 to 16; page 7, lines 5 to 7; page 16, lines 20 to 26; and page 18, lines 16 to 20 of the Priority Application.

l) "20-30 consecutive nucleotides in length"

Support for this language may be found, *inter alia*, at page 10, lines 15 to 17 of the Priority Application.

m) "a viral DNA polymerase gene, a viral RNA polymerase gene, a viral coat protein gene, or a visually-detectable gene involved in determining an external phenotype"

Support for this language may be found, *inter alia*, at page 10, lines 15 to 21 of the Priority Application.

n) "identical"

Support for this language may be found, *inter alia*, at page 3, lines 12 to 14; page 8, lines 14 to 22; and page 18, lines 17 to 18 of the Priority Application.

o) "inverted orientation"

Support for this language may be found, *inter alia*, at page 18, lines 16 to 20 of the Priority Application.

p) "repeating sequence"

Support for this language may be found, *inter alia*, at page 18, lines 16 to 20 of the Priority Application.

q) "stuffer fragment"

Support for this language may be found, *inter alia*, at page 19, lines 14 to 22 of the Priority Application.

Claim Interpretation

On page 5 of the November 3, 2008 Office Action, the Examiner has interpreted the configuration of Applicants' claimed double-stranded synthetic gene (currently amended to read "double-stranded DNA construct) set forth in the instant claims to be " a first structural gene sequence in the sense orientation relative to the target gene and a second structural gene sequence in the antisense orientation, making the second structural gene sequence complementary to the target gene." Applicants would like to clarify that each of the first and second "structural gene sequences" is comprised of double-stranded DNA. The Examiner's use of the word "complementary" in the above-referenced interpretation implies that the second structural gene sequence is single-stranded, which is incorrect. Claims 134 and 155 clearly describe a double-stranded DNA construct with a first double-stranded structural gene sequence, comprising 20-30 nucleotides in length identical in sequence to a region of a

target gene, and a second double-stranded structural gene sequence comprising 20-30 nucleotides in length identical in sequence to and in an inverted orientation relative to the first double-stranded structural gene sequence. Thus, the region of the resultant RNA derived from the first structural gene sequence will be in the sense orientation relative to the 20-30 nucleotides of the target RNA and the region of the resultant RNA derived from the second structural gene sequence will be in the antisense orientation and be complementary to the same 20-30 nucleotides of the target RNA.

Information Disclosure Statement

Applicants are concurrently submitting herewith three Supplemental Information Disclosure Statements, and respectfully request consideration of all items disclosed.

Claim Objections: 37 CFR § 1.75(c)

The November 3, 2008 Office Action objected to claims 146 to 149 under 37 CFR § 1.75(c) as allegedly of improper dependent form for failing to further limit the subject matter of claim 134.

In response, to clarify the invention, Applicants have hereinabove amended claim 134 to clarify that the length of the repeating sequence within the double-stranded DNA construct is only 20-30 nucleotides in length. For example, a double-stranded DNA construct within claim 134 would have a first structural gene sequence of 20 nucleotides and a repeated sequence, also of 20 nucleotides, inverted relative to the first structural gene sequence, with a stuffer fragment separating the two structural gene sequences. (*See, e.g.*, diagram "A", attached hereto as

Exhibit 2). Because the stuffer fragment is not a "repeating sequence" it is not included in the 20-30 nucleotide size limitation and could therefore be any length. The restriction to 20-30 nucleotides relates to the length of each of the first structural gene sequence and separately the second structural gene sequence forming a "repeating sequence" and does not limit the combined lengths of the first structural gene sequence, the stuffer, and the second structural gene sequence. Accordingly, claims 147 to 149 further limit the size of the stuffer fragment because they assign ranges of stuffer fragment lengths.

Similarly in response to the Examiner's remarks on page 3 of the November 3, 2008 Office Action with respect to claim 146. The interrupted palindrome is composed of the first structural gene sequence, the stuffer and the second structural gene sequence. The first and second structural gene sequences form a repeating sequence that is 20 to 30 nucleotides in length, and arranged as an inverted repeat. Therefore, the length of the sequence that is repeated is limited to 20 to 30 nucleotides and does not include the length of the stuffer.

Written Description Rejection: 35 U.S.C. § 112

The November 3, 2008 Office Action objected to claim 134, and dependent claims 135, and 142 to 154, under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement because the claims contain subject matter that was not described in the specification. The Examiner referred to page 10, lines 15 to 21 of the Priority Application, and states that the structural genes of 20 to 30 nucleotides are a preferred embodiment targeting specific genes, including viral DNA or RNA polymerases, viral coat proteins, or visually-

detectable genes involved in determining pigmentation, cell death or other external phenotypes. The Examiner purported that this section of the specification does not disclose the use of structural genes of 20 to 30 nucleotides to target any gene, but only to target the specific classes of genes named. Because of this assumed limitation, the Examiner alleged that the disclosure of the specification is not commensurate in scope with the claimed invention.

In response, Applicants respectfully traverse.

The claims are commensurate in scope with the disclosure of the specification. The claimed invention is a process for producing an RNA molecule to inhibit the expression of a "target gene." As stated in the specification, "the term 'target gene' shall be taken to refer to any gene, the expression of which is to be modified using the synthetic gene of the invention. Preferred target genes include, but are not limited to viral genes and foreign genes which have been introduced into the cell, tissue or organ or alternatively, genes which are endogenous to the cell, tissue or organ." (*See* page 7, lines 17 to 20 of the Priority Application). The claimed invention is aimed at inhibiting the expression of a "target gene" in mammalian cells. In the Examples described on pages 25 to 39 of the Priority Application, there are various examples of double-stranded DNA constructs that were designed to produce RNA that would inhibit the expression of a "target gene" in mammalian cells. The DNA constructs designed for this purpose were derived from the commercially available mammalian expression vectors pEGFP-N1, pCMVLacI, pOPRSVI/MCS and pSVL. (*See* pages 25 to 26 of the Priority Application). The passage referred to by the Examiner on page 10, lines 15 to 21 of the Priority Application, describes "target genes" in mammalian

cells and there is nothing in that passage that is not a target gene in a mammalian cell. The specific viruses named are all viruses that infect mammalian cells. Furthermore, the term "visually-detectable gene" is any gene whose influence is visually detectable. Products of a visually detectable gene include RNA or protein, which can be detected visually using various technologies including Northern Blots, Quantitative Real-Time PCR, Western Blots, or other biochemical assays. Thus, a "visually-detectable gene" is merely a term the specification uses for a "target gene" in a mammalian cell.

Accordingly, Applicants respectfully submit that the passage referred to by the Examiner defines the minimum length of structural gene components aimed at target genes in mammalian cells to be 20 to 30 nucleotides. Because support *in haec verba* is not required, the specification fulfills the written description requirement for claim 134, and dependent claims 135, and 142 to 154.

If the Examiner still believes that the specification is not commensurate in scope with claim 134, Applicants have added new claim 155, which recites verbatim the subject matter from page 10 of the Priority Application cited by the Examiner.

Claim Rejections Under 35 U.S.C. § 103(a) - Fire et al. Patent in view of in view of Agrawal et al., Gold et al., Kotin et al. and Chatterjee et al.

The November 3, 2008 Office Action rejected claims 134 to 154 under 35 U.S.C. § 103(a) as allegedly unpatentable over Fire et al. (US 5,605,559) in view of Agrawal et al. (WO 94/01550), Gold et al. (US 5,270,163), Kotin et al. (US 5,580,703) and Chatterjee et al. (US 5,474,935). The Examiner's specific rationale is set forth on pages 6 through 10 of the November 3, 2008 Office Action.

Fire et al. Patent is not prior art to the claimed invention

As Applicants pointed out previously, Fire et al. Patent is not prior art to the subject application. The amended claims herein are entitled to the priority of the March 20, 1998 filing date of Australian Provisional Patent Application No. PP2492. Fire et al. Patent issued from an application submitted to the United States Patent and Trademark Office on December 23, 1998, *i.e.* after the priority date of the subject application.

Fire et al. Patent claims the benefit of U.S. Provisional Application No. 60/068,562, filed December 23, 1997 ("Fire et al. Provisional"). However, Fire et al. Provisional discloses less than Fire et al. Patent. Applicants attach hereto as **Exhibit 3** a copy of Fire et al. Patent marked-up to show differences from Fire et al. Provisional. Any rejection which relies on disclosure not in Fire et al. Provisional is improper.

In the November 3, 2008 Office Action, the Examiner stated that

Applicants did not provide specific arguments that illustrated the relevance of the differences between the Fire et al. Provisional and the Fire et al. Patent. In response, Applicants point out the following relevant examples:

- On page 5, line 3, of the November 6, 2007 Office Action the Examiner stated "Fire et al. teach at columns 21-22 that a single promoter can be used to express an inverted duplication of a self-complementary dsRNA … ." This information is not disclosed in the Fire et al. Provisional (*See,* **Exhibit 3** difference 168).

- On page 7, line 3, of the November 3, 2008 Office Action the Examiner stated "At column 10 [of the Patent] Fire et al. teach that viruses can be targeted, including HIV." This information is not disclosed in the Fire et al. Provisional (*See,* **Exhibit 3** difference 132).

Therefore, this information was disclosed after the Applicants' priority date and cannot be relied upon in this obviousness-type rejection of Applicants invention.

I. Applicable Law for Determining Whether an Invention is Obvious

The determination of whether a claimed invention is obvious requires an analysis according to the framework of *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966) (attached hereto as **Exhibit 4**). *See,* M.P.E.P. § 804(II)(B)(1). The *Graham* analysis requires the following factual inquiries:

a. determine the scope and content of the combined teaching of the prior art;

b. determine the differences between the combined teaching of the prior art and the claims at issue;

c. determine the level of ordinary skill in the pertinent art; and

d. evaluate any objective indicia of nonobviousness (secondary considerations).

The Supreme Court of the United States most recently reaffirmed the *Graham* analysis in *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 U.S.P.Q.2d 1385, 1391 (2007) (attached hereto as **Exhibit 5**). The Supreme Court has continually cautioned against slipping into hindsight reconstruction. The *Graham* Court cautioned that it is necessary "to guard against slipping into use of hindsight and to resist the temptation to read into the prior art the teachings of the invention in issue." *Graham*, 383 U.S. at 36, 148 U.S.P.Q. at 474 (internal quotations omitted). The *KSR* Court reiterated the need for a fact finder to be aware "of the distortion caused by hindsight bias" and to "be cautious of arguments reliant upon ex post reasoning." *KSR*, 82 U.S.P.Q.2d at 1397.

Factors such as uncertainty and lack of predictability in the field at the time of the invention must be considered. *See, e.g. KSR*, 82 U.S.P.Q.2d at 1396. Even if there was a general suggestion or motivation to attempt to produce the invention, uncertainty and lack of predictability in the field will render the invention patentable and not obvious. *See*, M.P.E.P. §

2143.02; *In re Vaeck*, 947 F.2d 488, 495, 20 U.S.P.Q.2d 1438, 1444 (Fed. Cir. 1991); *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1207-08, 18 U.S.P.Q2d 1016, 1022-23, (Fed. Cir. 1991) (attached hereto as **Exhibits 6 and 7**, respectively) (Holding invention non-obvious even though it was "obvious to try" because lack of predictability in the biotechnology field eliminated reasonable expectation of success). Consideration of these factors is necessary when analyzing whether an invention is obvious; as the Supreme Court explained in *KSR*, one "must ask whether the improvement is more than the predictable use of prior art elements." *KSR*, 82 U.S.P.Q.2d at 1396 (emphasis added).

The analysis is the same regardless of where in the prior art the elements are disclosed. The patentability of a claim to a species or subgenus embraced by a single prior art generic disclosure should be analyzed no differently than any other claim for purposes of 35 U.S.C. 103. *See, e.g. Ortho-McNeil Pharmaceutical, Inc v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 86 U.S.P.Q. 1196 (Fed. Cir. 2008); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963); *In re Brouwer*, 77 F.3d 422, 37 U.S.P.Q.2d 1663 (Fed. Cir. 1996); *In re Ochiai*, 71 F.3d 1565, 37 U.S.P.Q.2d 1127, (Fed. Cir. 1995); *In re Baird*, 16 F.3d 380, 29 U.S.P.Q.2d 1550 (Fed. Cir. 1994); (attached hereto as **Exhibits 8 to 12**, respectively) and M.P.E.P. § 2144.08 (Rev. 6, Sept. 2007).

Finally, when determining whether a claimed invention is patentable, the relevant inquiry is not whether a particular difference between the prior art and the claims would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 U.S.P.Q. 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713

F.2d 782, 218 U.S.P.Q. 698 (Fed. Cir. 1983) (attached hereto as **Exhibits 13 and 14**, respectively). For example, the only report of RNA interference at the time was the Fire et al. Letter to Nature (*See*, Fire et al., Nature, 1998, attached hereto as **Exhibit 15**) in which Fire et al. described exogenously injecting a prepared dsRNA in *C. elegans*. Applicants' claims recite a process of endogenously expressing a DNA construct designed to produce in the nucleus of a mammalian cell a hairpin RNA. The combined effects of changing to endogenous production of a RNA different from that reported to work by Fire et al. in a different organism could not be predicted. Having no information about the mechanism of RNA interference, and knowing that Fire et al. concluded that "[a] simple antisense model is not likely" to explain his observations of RNA interference (*See*, Fire et al. Letter to Nature), one of ordinary skill in the art at the time had no basis to predict whether endogenous production in a mammalian cell as claimed would work.

As established by factual evidence from the relevant time, Applicants' claimed invention is more than merely the predictable use of prior art elements. Predictability in achieving a result specified in a patent claim through assembly of "known" components was a critical element of the Supreme Court's *KSR* decision. Indeed, the Court in *KSR* emphasized the importance of asking whether or not a particular combination of references would lead to a predictable solution to a problem. *See, e.g.*, *KSR*, 82 U.S.P.Q.2d at 1397. RNA interference and particularly its mechanism were a mystery at the time; as such there was hardly anything predictable about the selection of elements recited in the pending claims.

When ascertaining whether an invention is obvious, "[a]

factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 U.S.P.Q.2d 1385, 1397 (2007). Applicants respectfully submit and show herein that the obviousness rejections of record hint of hindsight bias and do rely on *ex post* reasoning.

II Application of the Controlling Legal Precedent to the Facts from the Relevant Time.

1. Scope and Content of the Prior Art

Fire et al. Provisional

The primary reference, the Fire et al. Provisional, reported that exogenous delivery of double-stranded RNA to cells by injection into the body cavity of *C. elegans* resulted in the inhibition of a target gene. The double-stranded RNA used by Fire et al. was produced outside of the organism as separate strands, subsequently annealed, and was sequence specific to the target over lengths from 299 to 1033 nucleotides.

The Fire et al. Provisional fails to describe a mechanism to explain the reported results. However, the Fire et al. Provisional explains at length that the mechanism, whatever it may be, is unrelated to known approaches for interfering with gene expression. Specifically, on pages 2-5, the Fire et al. Provisional discloses that the unknown mechanism is distinct from that of antisense interference, from that of triple-helix interference, and from that of co-suppression approaches.

Nonetheless, despite teaching that the mechanism underlying the

RNA interference phenomenon is different from all known phenomena at the time, the Fire et al. Provisional contains general boilerplate generalizing the disclosure of the reported results. Portions of the generic disclosure which are relevant for later analysis of differences recited in the pending claims are summarized below:

i) *Cell types.* Although the technique was only reported to work in *C. elegans*, the Fire et al. Provisional made a broad statement that the "cell with the target gene may be derived from or contained in any organism. The organism may [be] a plant, animal, fungus, or yeast" (page 11, lines 3 to 4) and goes on to list multiple species of plants, vertebrate animals, and invertebrate animals (page 11, lines 5 to 11).

ii) *Delivery methods.* The Fire et al. Provisional describes that the "RNA may be introduced directly into the cell (*i.e.*, intracellularly) or extracellularly." (Page 12, lines 1 to 2 of the Fire et al. Provisional). Importantly, "intracellularly," as used there, refers to introduction by injection as the needle delivers the RNA inside the cell as opposed to delivering RNA to the extracellular space or body cavity. The Fire et al. Provisional discloses multiple methods for introducing the RNA to the target cells, including "injection … , bombardment by particles covered by the RNA, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes with the RNA" (page 12, lines 7 to 10).

The Fire et al. Provisional discloses that "A viral vector packaged into a viral particle would accomplish both efficient introduction of an expression vector into the cell and transcription of RNA encoded by the expression vector" (page 12,

lines 10 to 12). This disclosure encompasses but does not describe a wide range of potential options including the following: (1) RNA retroviral vectors whose genomes are integrated into the host after reverse transcription and are expressed in the nucleus, including Murine leukemia virus and Lentiviruses;(2) single-stranded positive sense RNA virus vectors whose genes are expressed in the cytoplasm, including Sindbis virus, Semliki Forest virus, Poliovirus, and Kunjin virus; (3) single-stranded negative sense RNA virus vectors whose genes are expressed in the cytoplasm, including Influenza virus, Rabies virus, Vesicular stomatitis virus, and Sendai virus; and/or (4) double-stranded DNA virus vectors whose genes are expressed in the nucleus, including SV40 virus, Herpes Simplex Virus, Papillomavirus, Epstein Barr Virus, Adenovirus, Adeno-Associated virus and Baculovirus.

The Fire et al. Provisional does, however, teach that "[p]hysical methods of introducing" the RNA are "preferred". Page 12, line 7. Thus, the Fire et al. Provisional discloses all possibilities of known methods of introducing the RNA into a cell, and guides the reader that physical methods, i.e. not viral vector packaged into a viral particle type of methods, are the preferred methods for RNA interference.

iii) *Method of double-stranded RNA production.* Fire et al. disclose that the RNA used for RNA interference could be "chemically synthesized by manual or automated reactions" or be "synthesized by RNA polymerase of the cell or a bacteriophage RNA polymerase (e.g., T3, T7, SP6)" (page 11, lines 17 to 19). The RNA for the reported examples of Fire et al. Provisional was produced *in vitro* using phagemid clones with T3 and T7 promoters (page 15, line 5).

Each class of the foregoing options presented by the Fire et al. Provisional is not in any way connected to any other class of options. Other than the teaching of a preference for the use of physical delivery methods over other methods, no interrelationship is disclosed, for example, with regard to what type of delivery method could be used with which RNA molecule. The elements listed in the Fire et al. Provisional merely include substantially all the possible eukaryotic cell types, substantially all the common delivery methods for nucleic acids, and substantially all methods of producing RNA, that were known to one skilled in the art at that time.

Agrawal et al., Chatterjee et al., Gold et al., and Kotin et al.

Initially, Applicants point out that the Fire et al. Provisional is the only cited RNA interference reference. Two of the secondary references cited by the Examiner (Agrawal et al., and Chatterjee et al.) relate to antisense art. The relevance of their teaching is minimized by the teaching of the Fire et al. Provisional that the unknown mechanism of RNA interference is distinct from that of antisense. The other references, Gold et al. and Kotin et al., relate to protein biochemistry and virology, respectively, which are not in the scope of Applicants' invention. Thus, the combination of elements selected from the secondary references with the elements selected from the Fire et al. Provisional would be improper if it relies on current knowledge of the RNA interference mechanism.

Agrawal et al.

Agrawal et al. describe self-stabilized antisense oligonucleotides that comprise a target hybridizing region and a self-complementary region. *See* Agrawal et al. page 8, lines 22 to 25. The target hybridizing region is "preferably" from "about 8 to about 50 nucleotides in length" (*See* Agrawal et al. page 9, line 36 to page 10, line 1) and the self-complementary region is "about 4 or more base-pairs" but, in a preferred embodiment, "about 10 intramolecular base-pairs" (*See* Agrawal et al. page 15, lines 21 to 26). The self-complementary region could "involve every nucleotide of the oligonucleotide" and in this instance, the self-complementary region would be "about 50 nucleotides or less." *See* Agrawal et al. page 15, lines 26 to 30. Agrawal et al. teach that the "loop" formed by the self-complementary region should involve the "3'-most nucleotides" to protect the 3' end from endonucleases. *See* Agrawal et al. page 15, lines 20 to 26. Agrawal et al. teach that the oligonucleotides could be synthesized in vitro by chemical methods and they may contain modified linkages. *See* Agrawal et al. page 14, lines 11 to 35. The target hybridizing regions of the oligonucleotides could be complementary to nucleic acid sequences from viruses, pathogenic organisms, or cellular genes. *See* Agrawal et al. page 10, line 14 to page 13, line 4.

Chatterjee et al.

Chatterjee et al. describe the use of Adeno-associated virus (AAV) vectors, which integrate into the genome of the host, to express antisense oligonucleotides or dominant-negative proteins that will down regulate the expression of targeted viral or

cellular genes. Specifically, Chatterjee et al. target the 5' untranslated regions of human immunodeficiency virus (HIV) or herpes-simplex virus (HSV) RNA molecules. Chatterjee et al. also discuss possible mechanisms for antisense RNA including "the higher the molar ratio of antisense to sense transcripts, the greater the inhibitory effect" (Chatterjee et al. column 9, lines 29 to 31). This statement is discordant with the observations of Fire at al., further supporting the fact that antisense art and RNA interference occurred via two distinct mechanisms.

Gold et al.

Gold et al. describe a method to identify nucleic acid ligands for protein molecules. They transcribe DNA templates in vitro to produce single-stranded RNA, which contains randomized, contiguous regions of nucleotides for the purpose of interacting with proteins. In various figures of the specification, this randomized region corresponds to the loop of a RNA hairpin. The various RNAs are mixed with the target protein to promote RNA-protein interactions and the RNA molecules that bind are selected for another round of testing. They are amplified and the process is repeated. After a number of rounds of selection, Gold et al. are able to select for the sequence that most strongly interacts with the target protein.

Kotin et al.

Kotin et al. describe the isolation of a 4 kb long nucleic acid fragment that corresponds to the integration site of human adeno-associated virus (AAV). Also included within the fragment is a structural gene with a TATA-less promoter. Kotin et al. clearly state that this nucleic acid sequence can be "employed as a

probe, in conjunction with gene therapy, to identify successful integration of AAV" (*See* Kotin et al. column 2, lines 34 to 37). However, while Kotin et al. discuss the "potential advantages" of an AAV-based vector system, they do not describe an AAV-based vector and instead refer to the "proposed use" of AAV as a vector. (*See,* Kotin et al. column 2, lines 33 to 34).

In summary, none of the secondary references, even when combined with Fire et al., suggest the Applicants' claimed invention. As Fire et al. Provisional acknowledged, the antisense art could not contribute any information that would have assisted one of ordinary skill in the art to select the elements recited in the pending claims or to provide an expectation of such selection being successful. Furthermore, a discussion of nucleic acid ligands that act as protein inhibitors is irrelevant and knowledge of such technology would not have aided one of ordinary skill in the art in the development of the double-stranded DNA constructs for RNA interference described in the present claims.

2. Differences Between the Prior Art and the Pending Claims

The claimed invention is based on a selection of specific elements arranged in a manner not disclosed in the prior art. More specifically, the pending claims recite the results of the selection and combination of elements as summarized in the following list, and explained in detail thereafter:

i) *Endogenous delivery.* The constructs used in the methods as claimed are designed to deliver double-stranded RNA to the target cell by producing the RNA in the cell nucleus of mammalian cells. This approach differs from that reported to work by Fire et al., who produced double-

stranded RNA in vitro and then injected the RNA into *C. elegans* in either the cytoplasm of the target cell or an extracellular location outside the target cell. Although multiple options for introducing the RNA to the target cells are suggested in the Fire et al. Provisional, it is clear that the "physical methods of introducing" the RNA were preferred (*See,* Fire et al. Provisional page 12, lines 7 to 10). Chatterjee et al. use AAV vectors to express antisense RNA in the nucleus of target cells, but since the mechanism of antisense RNA differs from that of RNA interference, this information is irrelevant (as discussed above in section II-1. Kotin et al. discuss the "potential advantages" of AAV-based vectors, but never describe the construction or use of such vectors, let alone for inhibiting gene expression, and consequently teach even less than Chatterjee et al. Therefore, neither the Fire et al. Provisional, nor the cited references from the art at the time, either alone or in combination, provided a motivation to select endogenous delivery of the double-stranded RNA for RNA interference. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting the effects of such selection.

ii) *RNA hairpin structure.* Fire et al. recognized that "RNA structure was responsible for [its] inhibitory activity" (*See* page 14, lines 24 to 25) and they teach that double-stranded RNA composed of <u>two, separate</u> RNA strands is capable of inhibiting gene expression. Claim 134, the dependent claims thereon, and claim 155 recite methods that use double-stranded DNA constructs that would produce a <u>single</u> RNA strand in the nucleus designed to fold over onto

itself to form a duplexed hairpin structure. This structure differs from that of Fire et al.'s double-stranded RNA because it will have a loop of un-base-paired nucleotides on one end of the duplex. One of ordinary skill in the art at that time had no basis to select this different RNA structure. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting the effects of such selection on RNA interference.

The Examiner stated that Agrawal et al. teach a self-stabilized oligonucleotide, but the oligonucleotides of Agrawal et al. differ from those of the claimed invention. Agrawal et al. define two regions of a self-complementary oligonucleotide (the "target hybridizing region" and the "self-complementary region") and they suggest that intramolecular base-pairing could occur between "the target hybridizing region and the self-complementary region and/or by base pairing between complementary sequences within the self-complementary region" (*See,* Agrawal et al. page 8, lines 34 to 35 and page 9, lines 1 to 3). Neither of these options describes a hairpin RNA within which all of the base-paired nucleotides are part of the target hybridizing region, such as that produced by constructs recited in the claims of the present invention.

Agrawal et al. state that the base-pairing within the self-complementary region renders the oligonucleotides resistant to nucleolytic degradation (*See,* Agrawal et al. page 8, lines 32 to 35). However, because the oligonucleotides of Agrawal et al. function via antisense mechanisms, the self-

complementary oligonucleotides have to dissociate to expose the target hybridizing region to interact with the target sequence. Accordingly, the "self-complementary" region must dissociate in the presence of the target nucleic acid sequence. To do so, "the intermolecular base-paired structure formed by the hybrid between the target nucleic acid sequence and the target hybridizing region is more thermodynamically stable than the intramolecular base-paired structure formed by the self-complementary oligonucleotide." *See*, Agrawal et al. page 9, lines 12 to 17 of Agrawal et al. Although Agrawal et al. in one odd sentence mention that the intramolecular base-pairing could "involve every nucleotide of the oligonucleotide," a self-complementary oligonucleotide in which the entire target hybridizing region is involved with intramolecular base-pairing would dissociate less efficiently in the presence of the target sequence than a self-complementary oligonucleotide where only a portion of the target hybridizing region is involved in the intramolecular base-pairing. This would have been readily recognized by one of ordinary skill in the art at the time.

Consequently, one of skill in the art would not design a fully self-complementary oligonucleotide for the purpose of antisense-based inhibition of gene expression according to Agrawal et al. In fact, once the intramolecular base-pairing exceeds about 20 nucleotides in length, one of ordinary skill in the art would understand that the molecule would not disassociate at an acceptable rate under physiological conditions to effectively bind to the target. (*See*, Wallace R.B. et al., Nucleic Acids Res., 1979, attached hereto as **Exhibit 16**). Consistent with such

understanding, Agrawal et al. disclose their "preferred embodiment" to have only "about 10 intramolecular base-pairs formed in the self-stabilized oligonucleotide" (*See* Agrawal et al. page 15, lines 23 to 26).

Of course, the pending claims herein require 20 to 30 nucleotides. Selecting a molecule where intramolecular base-pairing involves "every nucleotide of the" 20 to 30 nucleotides, would render the disclosure of Agrawal et al. unsatisfactory for its intended purpose and therefore cannot be an obvious selection. *See, e.g., In re Gordon,* 733 F.2d 900; 221 U.S.P.Q. 1125 (Fed. Cir. 1984) (attached hereto as **Exhibit 17**).

The hairpin RNA produced by the claimed invention functions by a mechanism not disclosed by Agrawal et al. and is not subject to the same functional restrictions as the oligonucleotides of Agrawal et al. Importantly, this was not known prior to the filing of the subject application, making the selection of a fully complementary hairpin RNA even less obvious.

The Examiner also referred to Gold et al. as teaching RNAs with stem loop structures. However, the RNAs taught by Gold et al. are used to determine nucleotide sequences that provide an optimal motif for targeted protein interaction. The RNAs are not used to specifically inhibit gene expression. Furthermore, in the exemplified hairpins of Gold et al., the loop regions of the hairpins contain the "target regions" that are presumed to interact with the target proteins. This design differs from that of the Applicants' invention, where the "target region" is part of

the stem of the hairpin. The sequences in the loop regions of the RNAs of Gold et al. are randomized in order to optimize the sequence for protein binding, while the sequences in the stem region are held static. This is in direct contrast to the Applicants' RNA hairpins, where the targeting regions, located in the stem region, may be varied per given target. Consequently, even if Fire et al. and Gold et al. could properly be combined, the combination of Fire et al. with Gold et al. would have led one of ordinary skill in the art to place the targeting region in the "loop" of a hairpin, which would not result in a double-stranded RNA targeting region and would not lead to Applicants' invention. Additionally, the "loop" of Gold et al. most definitely does not read on the "stuffer" of Applicants' invention. The function of the "stuffer" is to allow the complementary regions of the RNA to efficiently base-pair with one another, while the "loop" of Gold et al. exists to present a single-stranded nucleotide binding site to a protein.

Thus, the combination of Fire et al., who do not explicitly claim double-stranded RNAs with a stuffer fragment, and Agrawal et al., who teach antisense oligonucleotides, and Gold et al., who teach nucleic acid ligands, would not lead one of ordinary skill in the art to produce a double-stranded DNA construct with a stuffer fragment that produces a double-stranded hairpin RNA for RNA interference. Neither Chatterjee et al., nor Kotin et al., teach the expression of double-stranded RNA.

iii) *Length of the double-stranded RNA.* Fire et al. reported the successful use of double-stranded RNA that was

299 to 1033 nucleotides long. The minimum length of the double-stranded or hairpin RNA produced by the constructs of the claimed invention is 20 to 30 nucleotides long. Although one of ordinary skill in the art may have been educated from the teachings of references from the antisense art, such as those of Agrawal et al., where small, not fully duplexed oligonucleotides were used, there was no teaching prior to the filing of the subject application to support the notion that double-stranded RNA shorter than 299 nucleotides long could cause RNA interference. Thus, the selection to reduce the size of the double-stranded RNA duplex by an order of magnitude was a great departure from what was reported by Fire et al. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting the effects of such selection.

iv) *Change to mammalian cells.* Fire et al. described RNA interference in *C.elegans.* They were able to inhibit the gene expression of a specific target gene by exogenously delivering double-stranded RNA that was 299 to 1033 nucleotides long to *C. elegans* cells. Applicants' invention calls for the expression of double-stranded RNA in mammalian cells. At the time of the subject invention, it was known in the art that double-stranded RNA caused a non-specific response in mammalian cells that resulted in cytotoxicity. Based on this response in mammalian cells, it was thought that "[a] similar mode of action would not be suspected to occur in mammals" for RNA interference. (Wagner R.W. and Sun L., Nature, 1998, attached hereto as **Exhibit 18**). Thus, prior to Applicants' invention it would

not have been possible to predict if RNA interference would work in mammalian cells. In fact, based on the knowledge in the art at the time, it was more probable that it would not work in mammalian cells.

Uncertainties of the selections made by the present invention.

At the infancy of RNA interference, Applicants' invention was a significant deviation from what was known to have a RNA interference effect. At that time, the mechanism underlying the effects reported by Fire et al. was a mystery and one skilled in the art had no framework within which to even rationally consider, much less predict, what effect any given change would have on the observations reported by Fire et al. Substantial evidence from the relevant time indicates that it was impossible to predict the effects of any change to that system. Proceeding with the selections made by the inventors of the subject application was fraught with uncertainties; the selections introduced numerous variables that could have impacted the function of Applicants' invention. Yet, despite the uncertainties, Applicants proceeded contrary to the limited expectations of the time.

i) The Selection of Endogenous Delivery Presented a Number of Unknowns.

- *Duplex RNA might not have gotten out of the nucleus.*

At the time of Applicants' invention, the only knowledge in the art regarding the intracellular location where RNA interference was happening was taught by Fire et al., who disclosed that efficient gene silencing occurred when double-stranded RNA was injected into the body cavity or cytoplasm of *C. elegans*.

Therefore, based on Fire et al., it would not be obvious that duplex RNA produced in the nucleus would efficiently translocate across the nuclear membrane to elicit the same inhibition observed by Fire et al. It was known to those of ordinary skill in the art that multiple proteins interacted with single-stranded mRNA to mediate its translocation through the nuclear pore. It was possible that the canonical export machinery would not have recognized duplex RNA to promote its egress from the nucleus. Alternatively, there may have been nuclear retention factors that could bind the duplex RNA to prevent it from leaving the nucleus.

In the November 3, 2008 Office Action, the Examiner discusses that viral vectors could be used to introduce exogenous sequences into the genome of a cell. Kotin et al. generally discuss that AAV-based vectors could be used in human gene therapy. Chatterjee et al. use AAV vectors to express antisense RNA targeted to the 5′ UTR of HIV. However, the production of antisense RNA in the nucleus is irrelevant because the claimed invention results in the production of duplex RNA in the nucleus. Chatterjee et al. did not demonstrate the production of duplex RNA in the nucleus. As already discussed, the characteristics of antisense RNA are not applicable to RNA interference. As Fire et al. admitted, "A simple antisense model" could not explain what they observed (Fire et al. Nature. 1998).

Nonetheless, antisense RNA may not even have to exit the nucleus to promote its inhibitory effects, which may include "transcriptional regulation, inhibition of splicing, inhibition of mRNA transport, and induction of mRNA instability" (Kumar M. and Carmichael G., Microbiol. Mol. Biol. Rev., 1998, attached hereto as **Exhibit 19**). In one example from the antisense RNA art, antisense RNA produced in the nucleus resulted in the

nuclear retention of duplex RNA formed with the target sense strand (Kim S. and Wold B.J., Cell, 1985, attached hereto as **Exhibit 20**). This example is illustrative in two respects: (1) it demonstrates that antisense RNA does not have to leave the nucleus to function; (2) it suggests that duplex RNA can get trapped in the nucleus. Consequently, the Examiner's argument that functional antisense RNA could be produced from an expression construct is irrelevant to the uncertainty of producing duplex RNA in the nucleus. Furthermore, one skilled in the art would have to question whether duplex RNA could get out of the nucleus.

- *Duplex RNA produced in the nucleus might be modified.*

The nucleus is a specialized compartment of the cell and contains factors that may only interact with macromolecules produced inside the nucleus. One example of such a factor is a nuclear double-stranded RNA dependent adenosine deaminase. In the nucleus, these enzymes target double-stranded RNA portions of duplexes and convert adenosine (A) to inosine (I), which makes the duplex unstable and may lead to unwinding and increased degradation (Kumar M. and Carmichael G., Microbiol. Mol. Biol. Rev., 1998, attached hereto as **Exhibit 19**). One of skill in the art would recognize that unwinding of the duplex would abrogate silencing function, based on the teaching of Fire et al. that the double-stranded character was important for function in RNA interference. Additionally, the incorporation of inosine in the RNA would decrease the stringency of the intramolecular base-pairing within the duplex, which could result in a heterogeneous collection of imperfect duplexes in the nucleus. Because one skilled in the art could not predict the effect that inosines would have on RNA interference, it would be difficult to predict if RNA duplexes created in the nucleus could mediate gene

silencing.

- *Polyadenylation may interfere with RNA interference.*

Messenger RNA precursors (pre-mRNA) that are produced in the nucleus are modified at their 3'terminus by the addition of a polyadenylation signal (poly-A tail) of ~200-250 adenine residues (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit 21**). At the time of the present invention, two proposed functions of the poly-A tail were: (1) to protect the transcript from degradation (Sachs A. and Wahle E. J. Biol. Chem. 1993, attached hereto as **Exhibit 22**); and (2) to stimulate transportation out of the nucleus (Huang Y. and Carmichael G., Mol. Cell. Biol., 1996, attached hereto as **Exhibit 23**). Therefore it was understood to be important for RNA produced in the nucleus to have a polyadenylation tail for protection and for transport out of the nucleus, and the Applicants discuss the inclusion of a poly-A tail in the Provisional on page 22, lines 12 to 30. However, one skilled in the art would not have been able to predict the effect that a poly-A tail would have had on the ability of an RNA duplex to mediate RNA interference. The poly-A tail would lead to a large, single-stranded overhang on both strands of the RNA duplex produced in the nucleus. Because the RNA duplexes of Fire et al. did not have a poly-A tail, it was not possible to predict how this structure would affect the RNA interference function of the RNA duplex resulting from the present invention.

- *Binding of heterogeneous nuclear ribonucleoproteins may affect duplex formation.*

When RNA is produced in the nucleus, it is quickly bound by numerous heterogeneous nuclear ribonucleoproteins (hnRNPs). One function of these proteins is to promote the correct processing

of endogenous pre-mRNA by preventing the formation of secondary structures, such as folding. (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit 21**). Thus, as RNA of the present invention is transcribed in the nucleus, hnRNPs could bind to the RNA and prevent it from folding to form a duplex. As stated above, Fire et al. considered the duplex structure of the injected RNA to be essential for RNA interference. Thus, the presence of hnRNPs in the nucleus could hinder the induction of RNA interference by inhibiting the formation of duplex RNA in the nucleus.

- *Binding of heterogeneous nuclear ribonucleoproteins may affect RNA interference function.*

Additionally, it is known that some hnRNPs from the nucleus remain associated with mRNA as it is translocated into the cytoplasm (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit 21**). Even if the RNA was able to assume a duplex structure, but the nuclear proteins remained bound to the RNA duplex when it encountered the silencing targets or unknown effectors in the cytoplasm, the bound duplex might not have been able to function properly to cause the interference.

ii) The Change to Hairpin RNA Presented Additional Unknowns

- *Hairpin RNA may be susceptible to nucleus specific ribonucleases.*

At the time of the present invention, a major concern with introducing RNA into cells was the degradation of that RNA. Assuming that the RNA of the present invention could form a hairpin in the nucleus, the hairpin would be expected to encounter nuclear double-stranded RNA ribonucleases (RNAses), such as RNAse III (Wu H. et al., J. Biol. Chem., 1998, attached

hereto as **Exhibit 24**). These enzymes would be expected to specifically degrade the hairpin RNA in the nucleus, but would not be expected to degrade hairpin RNA directly introduced into the cytoplasm by Fire et al. If hairpin RNA structures were degraded in the nucleus, then the RNA interference reported by Fire et al. would not occur.

iii) The Change to Shorter RNA Duplexes Presented Yet More Unknowns

Fire et al. showed that double-stranded RNA that was 299 nucleotides long was capable of efficiently causing RNA interference in *C. elegans.* Claim 134, the claims dependent thereon, and claim 155 recite use of DNA constructs designed to produce a dsRNA or a hairpin RNA that is 20-30 nucleotides long. Because the mechanism of RNA interference was unknown at the time, there was no indication prior to the filing of the subject application that the use of dsRNA or hairpin RNA an order of magnitude shorter than that shown to work by Fire et al. would cause the same result. The partial hairpin antisense molecules, e.g. of Agrawal et al., while shorter than the molecules of Fire et al. further confound the issue because Fire et al. clearly teach that knowledge from the antisense art cannot be applied to RNA interference (Fire et al., Nature, 1998). Recognizing this, one of ordinary skill in the art would be very concerned whether short hairpins would cause RNA interference.

In fact, those of more than ordinary skill, e.g. the inventors listed on the Fire et al. Provisional, raised the issue in published statements. *See, e.g.,* Tabara H., Grishok A., and Mello C., Science 1998, attached hereto as **Exhibit 25**. ("In most genes, any RNA segment of about 200 to 1000 nucleotides or

greater appears to be capable of inducing interference." "And controlled studies to determine the minimum length and the minimum sequence similarity to induce interference have yet to be reported and are likely to vary for different genes.").

Clearly, prior to the filing of the subject application, the consequence of decreasing the length of duplex RNA intended for gene silencing were unpredictable.

iv) The Change to Expressing Double-stranded RNA in Mammalian Cells.

At the time of the subject invention, it was well known in the art that double-stranded RNA stimulated a non-specific mechanism in mammalian cells that led to the global inhibition of translation and transcript degradation. Double-stranded RNA induces interferon production in mammalian cells, which causes the up-regulation of two double-stranded RNA response enzymes: RNA-regulated protein kinase (PKR) (also referred to as DAI) and 2',5' oligoadenylate synthetase (also called 2',5'-oligo(A) polymerase). Direct activation of PKR by double-stranded RNA leads to the inhibition of translation (Clemens M., Int. J. Biochem. Cell. Biol., 1997, attached hereto as **Exhibit 26**). Double-stranded RNA also leads to the activation of 2',5' oligoadenylate synthetase, which catalyzes the production of 2',5' oligoadenylates. These molecules activate the ribonuclease RNAse L, which leads to the non-specific degradation of single-stranded RNA transcripts (Jacobs B.L. and Langland J.O., Virology, 1996, attached hereto as **Exhibit 27**). Studies described in the art at the time of the present invention maintained that double-stranded RNA had to be longer than 30 nucleotides to bind and activate either PKR or 2',5'

oligoadenylate synthetase, and optimal binding occurred as the double-stranded RNA approached lengths of about 80 nucleotides. (Manche L. et al., Mol. Cell. Biol., 1992, attached hereto as **Exhibit 28**, and Minks M.A. et al., J. Biol. Chem, 1979, attached hereto as **Exhibit 29**). Thus, using the lengths of double-stranded RNA taught by Fire et al. to cause RNA interference in mammalian cells would have lead to a non-specific response resulting in cellular shutdown. Furthermore, it could not be predicted if reducing the length of the double-stranded RNA to below 30 nucleotides would result in RNA interference, as discussed above in Section (iii).

Antisense art is non-analogous to RNA interference art and its combination with Fire et al. Provisional is improper.

The Examiner's proposal to combine teachings from antisense art with the Fire et al. Provisional is illogical in the face of the clear teaching in the Fire et al. Provisional that RNA interference operates by a distinct mechanism. *See, e.g.,* pages 2 to 5 of the Fire et al. Provisional. Consequently, the effects of such a combination cannot be predicted.

It is also important to recognize that the Examiner is picking and choosing elements for purposes which have been acknowledged by Fire et al. to not be involved in RNA interference. The Examiner's proposal to combine teachings from antisense art with the Fire et al. Provisional is illogical in the face of the clear teaching in the Fire et al. Provisional that their invention operates by a distinct mechanism. *See, e.g.,* pages 2 to 5 of the Fire et al. Provisional. The effects of such a combination cannot be predicted.

The Examiner has alleged that claim 134 and the claims dependent thereon are obvious over the Fire et al. Provisional as a primary reference, in view of Agrawal and Chatterjee. As noted above, Fire et al. is the first publication of experiments describing observations that became known as RNA interference. Each of Agrawal and Chatterjee expressly relate to "antisense" technology. However, in regard to a possible mechanism for RNA interference, Fire et al. explicitly acknowledge that "[a] simple antisense model is not likely: annealing between a few injected RNA molecules and excess endogenous transcripts would not be expected to yield observable phenotypes." *See,* Fire et al. Letter to Nature; and pages 2-5 of the Fire et al. Provisional. Therefore, combining elements from these acknowledged distinct fields is improper.

Nonetheless, the Examiner erroneously turns to the antisense art to attempt to supplement the teachings of Fire et al. to demonstrate that the appropriate knowledge did exist in the art at the time of Applicants' invention to render the instant invention obvious. For example, the Examiner references the teachings of Agrawal et al. to provide evidence that knowledge of hairpin RNAs with terminal loops existed in the art at the time of the invention. However, the function of the loops disclosed in that reference differs from that of the stuffer in the Applicants' invention. The loops of the antisense oligonucleotides in Agrawal et al. are present to protect the oligonucleotides from degradation in the cells. This differs from the purpose of the "stuffer fragment", which permits complementary base-pairing between the sense and the antisense strands of the hairpin, which are the regions that correspond to the target sequence.

Additionally, the Examiner cites a teaching from Chatterjee et al. that antisense oligonucleotides targeting the "areas of critical viral RNA transcripts including the 5'-untranslated region, splice sites, and the polyadenylation signal have demonstrated significant antiviral activities." *See*, Chatterjee et al. column 3, lines 11 to 15. At the time, one proposed mechanism known in the art for antisense inhibition was a physical blockage of the translation machinery that resulted from the hybridization of the antisense oligonucleotide to the 5' untranslated region or the translation initiation codon. Because the mechanism of RNA interference was not known at the time, it was impossible to predict that structural gene regions targeted to the 5' untranslated region would be effective.

3. The Level of Ordinary Skill in the Art at the Time

It is imperative that the obviousness inquiry proceed based on what would have been obvious to a person of ordinary skill in the art at the time, not based on what is obvious to a judge, to an examiner, to a layman or to geniuses in the art then or now. The factors that are considered in determining the level of ordinary skill in the art include: 1) the education level of the inventor, 2) the types of problems encountered in the art, 3) the prior art solutions to those problems, 4) the rapidity with which innovations are made, 5) the sophistication of the technology, and 6) the educational level of the technical workers in the field. *Environmental Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693, 697 (Fed. Cir. 1983), cert. denied, 464 U.S. 1043 (1984) (attached hereto as **Exhibit 30**).

Applicants respectfully submit that as of March 20, 1998, a person of ordinary skill in the art related to the subject matter

at issue herein would have been a person with a Ph.D. degree in microbial genetics, biochemistry, molecular biology or a related discipline with postdoctoral research experience in the field of recombinant DNA technology or a physician with equivalent educational and laboratory research experience in the same field.

Individuals of ordinary skill at the time readily acknowledged that experiments designed to shed light on the possible mechanism of RNA interference "painted an even more mystifying picture" (Wagner R.W. and Sun L., Nature, 1998, attached hereto as **Exhibit 18**). Certainly those of ordinary skill in the art at the time recognized the multiple unknowns involved in trying to achieve RNA interference using endogenous delivery of hairpin RNA as discussed above. Because of this unpredictability in the nascent field at the time, one of ordinary skill could not find the claimed invention obvious.

4. Unexpected Results

The constructs recited by the pending claims herein have properties which could not have been predicted from the prior art. The attainment of unpredictable results is a clear indication of non-obviousness. *See, e.g. KSR* at 1739-40. Despite the multitude of uncertainties associated with the claimed selection of elements, the selection disclosed in the subject application has been shown to be effective.

The success of RNA interference in mammalian cells, using double-stranded RNA of 22-24 nucleotides, was considered "particularly striking" due to the non-specific effects previously observed in these cells because of the double-stranded RNA response. Caplen N.J., Proc. Natl. Acad. Sci. USA, 2001, attached hereto as

Exhibit 31. The efficacy of such an approach came as a surprise to those skilled in the art, as illustrated by comments such as, "[m]ore surprising was the finding that DNA constructs encoding … blunt-ended duplexes with up to 29 base pairs were able to mediate RNA interference." Tuschl T., Nature Biotechnology, 2002, attached hereto as **Exhibit 32.** The constructs being referred to were RNA hairpins that were produced from a double-stranded DNA construct with 27 or 29 base-pairs of "structural gene sequence" that was specific to a target gene. Paddison P.J. et al., Genes & Development, 2002, attached hereto as **Exhibit 33.**

Furthermore, it was reported that endogenous delivery of hairpin RNA "dramatically reduced" expression of a target gene more efficiently than endogenous delivery of non-hairpin dsRNA. Yu J.Y. et al., Proc. Natl. Acad. Sci. USA 99, 2002, attached hereto as **Exhibit 34.** Yu et al. did not expect such a high level of efficiency of reduction of expression of a target gene following the endogenous delivery of hairpin RNA, else Yu et al. would not have characterized the reduction as "dramatic". The increased efficiency of endogenous delivery of hairpin RNA over exogenous delivery of hairpin RNAi as exemplified by Yu et al. was unpredictable from Fire et al. Provisional.

It should be noted that Yu et al. used U6 promoters to produce hairpin RNA in the nucleus. It was well known in the art that the RNA produced in this manner was commonly retained in the nucleus. *See* Noonberg SB et al., Nucleic Acids Research, 1994, attached hereto as **Exhibit 35**; and Good PD et al., Gene Therapy, 1997, attached hereto as **Exhibit 36**). This knowledge only accentuates the unpredictability of whether endogenous delivery of RNA would work, as discussed at length above. Paul et al. acknowledged these concerns when they stated, "Another unexpected

finding was that the expression from the U6 snRNA promoter cassettes, which give primarily nucleoplasmic expression, would succeed in inhibiting target expression when a majority of existing mRNA is cytoplasmic." Paul et al., Nature Biotechnology, 2002, attached hereto as **Exhibit 37**). The concerns of Paul et al. were aligned with the teachings of Fire et al., who taught that the double-stranded RNA needed to be in the cytoplasm to cause RNA interference. Thus, Noonberg et al. and Good et al. support Applicants' discussion that the results of the claimed selection could not have been predicted from teachings of the prior art.

In conclusion, prior to the filing of the subject application, the effectiveness of endogenously produced RNA was unexpected from the Fire et al. Provisional.

5. Secondary Considerations

Beyond the analysis of whether a proper *prima facie* case of obviousness is present, evidence of objective criteria showing nonobviousness must be considered. Specifically, skepticism of experts at the time is significant and respected objective evidence of nonobviousness. Such evidence is not cumulative in the obviousness analysis, but rather "constitutes independent evidence of nonobviousness." *Ortho-McNeil Pharmaceutical, Inc v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 86 U.S.P.Q. 1196 (Fed Cir. 2008), citing *Catalina Lighting, Inc. v. Lamps Plus, Inc.*, 295 F.3d 1277, 1288 (Fed. Cir. 2002); *Pharmastem Therapeutics Inc. v. Viacell, Inc.*, 491 F.3d 1342; *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc.*, 471 F.3d 1369 (attached hereto as **Exhibits 8 and 38 to 40**, respectively).

Thus, the skepticism of experts, including the skepticism of the inventors named on the Fire et al. Provisional, about the selection of elements recited by the pending claims is highly probative and provides an "independent" basis for finding the pending claims patentable.

i) *Skepticism in the art at the time of the invention.*

As previously discussed, the mechanism of RNA interference was unknown at the time of the instant invention. The Fire et al. Letter to Nature was the only report of RNA interference in the art at that time (attached hereto as **Exhibit 15**). The results reported by Fire et al. were so unexpected, there was at the time general skepticism about the reported results themselves, and extreme skepticism of what sort of modifications the reported experiment would tolerate. The impressions of those in the art were evident through published statements that referred to the difficulties and uncertainties associated with the initial RNA interference studies. The following are a sampling of such statements, many of which were made by the inventors named on the Fire et al. Provisional:

- "[T]he lack of a clear understanding of the critical requirements for interfering RNA led to a sporadic record of failure and partial success." Fire et al. Provisional, page 13, line 29 and page 14, line 1.

- Experiments designed to shed light on the possible mechanism of RNA "painted an even more mystifying picture." Wagner R.W. and Sun L., Nature, 1998, attached hereto as **Exhibit 18**.

- "Whatever the mechanism might be, dsRNA-mediated inhibition of gene expression will provide a useful alternative for working out gene function in *C. elegans* and, maybe, in other animals and plants." (Emphasis added.) Wagner R.W. and Sun L., Nature, 1998, attached hereto as **Exhibit 18**.

- "Controlled studies to determine the minimum length and the minimum sequence similarity to induce interference have yet to be reported and are likely to vary for different genes." Tabara H. et al., Science, 1998, attached hereto as **Exhibit 25**.

- "A similar mode of action would not be suspected to occur in mammals." Wagner R.W. and Sun L., Nature, 1998, attached hereto as **Exhibit 18**.

- "Any gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR." Montgomery M. and Fire A., Trends in Genetics, 1998, attached hereto as **Exhibit 41**.

It is apparent from these quotes that at the relevant time there was significant skepticism about more minor modifications to the reported results then the modifications resulting from the selection as claimed in the subject application. Inventors of the claimed invention proceeded contrary to expectations at the relevant time to face the multiple obstacles published by many and arrived at the claimed invention. Proceeding contrary to accepted wisdom at the time is, of course, inventive.

The above discussion unambiguously shows that the pending claims are non-obvious in view of Fire et al., even when combined with Agrawal et al., Chatterjee et al., Gold et al. and Kotin et al., and should be allowed without further delay. Such action is respectfully requested. The obviousness rejections of the November 3, 2008 Office Action are inappropriate.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee other than the enclosed extension of time fee of $1,110.00 is deemed necessary in connection with the filing of this Amendment. However, if any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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